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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat **GUIDELINE:** 83-3(a)

P.C. CODE: 030001 **TOX. CHEM. No:** 315

TEST MATERIAL: **2,4-Dichlorophenoxy acetic acid (2,4-D)**

CITATION: Nemec, M.D, Tasker, E.J. Werchowski, K.M, and Mercieca, M.D . *" A TERATOLOGY STUDY IN FISCHER 344 RATS WITH 2,4-DICHLOROPHENXOYACETIC ACID"*. WIL Research Laboratories, Inc. Study No. WIL-81135, March 2, 1983. **Accession No. 000251031.** Unpublished.

REGISTRANT: Industry Task Force on 2,4-D Research Data.

EXECUTIVE SUMMARY: In a developmental toxicity study (Acc. # 000251031) pregnant Fischer-344 rats (35/group) were given oral administration (gavage) of 2,4-dichlorophenoxy acetic acid (technical, 97.5%) in corn oil at 0 (vehicle control), 8, 25, or 75 mg/kg/day during gestation Days 6 through 15, inclusive.

Treatment did not affect survival, induce clinical signs or maternal wastage, cause body weight changes, or alter reproductive parameters. Maternal toxicity was limited to decreases in body weight gain in dams at 75 mg/kg/day; when compared to the vehicle control, the decreases were -43%, -21% and -2% for gestation days 6-10, 6-15, and 0-20, respectively. Although these decreases were not statistically significant, they were considered to be treatment-related because decreases in body weight gain was also seen in a 2-generation reproduction toxicity study in the same strain (Fischer 344) of rats at a comparable dose of 80 mg/kg/day (actual dose = 75 mg/kg/day). **Based on these findings, for maternal toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.**

Table 5. Embryotoxicity and Fetotoxicity Observed in Sprague-Dawley Rats.[a]

ANOMALIES	Vehicle Control		87.5 mg/kg/day	
INCIDENCE (%)	Fetus	Litter	Fetus	Litter
Subcutaneous edema	8/242 (3)	8/41 (20)	31/117 (26)*	15/19 (79)*
Delayed ossification of sternebrae	98/245 (40)	31/41 (76)	31/119 (26)	10/19 (53)
Sternebrae with split centers of ossification	35/245 ((14)	19/41 (46)	48/119 (40)*	11/19 (58)
2nd, Wavy ribs	0/245 (0)	0/41 (0)	6/119 (5)*	2/19 (11)
Lumbar ribs	5/245 (2)	3/41 (7)	10/119 (8)*	4/19 (21)
Missing sternebrae	0/245 (0)	0/35 (0)	8/119 (7)*	5/19 (26)*

a = Data obtained from Schwetz et al. 1971.

C. Developmental Toxicity in Rabbits: In a parallel study (MRID # 41747601; HED Doc.# 008462), artificially impregnated New Zealand rabbits (20/dose) were given oral administration of 2,4-D acid in 0.5% methylcellulose at 0, 10, 30 or 90 mg/kg/day during gestation days 6 through 18. Maternal toxicity was limited to the high dose and was based on ataxia, abortion, slight nonsignificant decreases in body weight gain during the dosing and post dosing period, and a nonsignificant reduction in corrected body weight gain during the entire period. No developmental toxicity was seen at any dose level. No external, visceral, or skeletal anomalies (malformations or variations) were seen in any of the fetuses. For maternal toxicity the NOEL was 30 mg/kg/day and the LOEL was 90 mg/kg/day, For developmental toxicity, the NOEL was 90 mg/kg/day and the LOEL was > 90 mg/kg/day.

V. CONCLUSIONS

Under the conditions of this study, the following NOELs and LOELs are established:

Maternal toxicity NOEL = 25 mg/kg/day
Maternal toxicity LOEL = 75 mg/kg/day
Developmental toxicity NOEL = 25 mg/kg/day
Developmental toxicity LOEL = 75 mg/kg/day

This developmental toxicity study in rats is classified as **Acceptable** and satisfies the Subdivision F Guideline requirement for a developmental toxicity study in rats [83-3(a)].

IV. DISCUSSION

1. INVESTIGATOR'S CONCLUSIONS: The authors concluded that the inhibition of body weight gain (-21 to -43%) observed at the high-dose (75 mg/kg/day) during the dosing period (Days 6-15) as slight maternal toxicity since mean values never attained control values for the period corresponding to treatment. It was also concluded that treatment had no effect on any of the cesarean parameters and that 2,4-D was not a teratogen in rats.

2. REVIEWERS DISCUSSION

   A. Maternal Toxicity

   Body Weight: When compared to the vehicle control, maternal body weight gains of dams at 75 mg/kg/day were decreased by -43%, -21% and -2% for gestation days 6-10, 6-15, and 0-20, respectively. Although these decreases were not statistically significant, they were attributed treatment-related because decreases in body weight gain were also seen in a 2-generation reproduction toxicity study (MRID No. 25944206) in the same strain (Fischer 344) of rats at a comparable dose of 80 mg/kg/day (actual dose ≈ 75 mg/kg/day). Based on these findings, for maternal toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.

   B. Developmental Toxicity: The increases in skeletal variation observed at 75 mg/kg/day (presence of 7th cervical and 14th rudimentary ribs, malaligned sternebrae, reduced ossification of the vertebral aches, and unossified sternebrae) were not statistically significant when compared to the vehicle control nor did they show dose-response. However, they were considered to be treatment related since some of the variations (malaligned sternebrae, 14th rudimentary ribs and reduced ossification of vertebral arches) seen in this study were also seen in the F_{1b} pups of dams fed 2,4-D at 80 mg/kg/day (actual dose, ≈ 75 mg/kg/day) in the 2-generation reproduction study in the same strain of rats (Fischer 344).

   In addition, as shown below in Table 5, skeletal variations of the ribs (2nd wavy ribs, lumbar ribs) and missing sternebrae were also seen in a teratology study using a different strain (Sprague-Dawley) of rats at a comparable dose of 87.5 mg/kg/day. In that study, pregnant Sprague-Dawley rats were given oral administration of 2,4-D acid at 0, 12.5, 25, 50, 75 or 87.5 mg/kg/day during gestation days 6 through 15 (Schwetz *et al.*, 197).

   Thus, based upon a weight-of-evidence from the reproduction and developmental toxicity studies in Sprague-Dawley and Fischer 344 rats, it is concluded that developmental toxicity did occur at the high dose (75 mg/kg/day) in this study. Based on these findings, for developmental toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.

B. Developmental Toxicity

1. External Examination: No treatment-related external anomalies were seen in any of the 262, 276, 269 and 250 fetuses examined at 0, 8, 25 or 75 mg/kg/day, respectively.

2. Visceral Examination: No treatment-related visceral anomalies were seen in any of the 131, 140, 136 and 123 fetuses examined at 0, 8, 25 or 75 mg/kg/day, respectively.

3. Skeletal Examination: No treatment-related skeletal malformations were seen in any of the 131, 140, 136 and 123 fetuses examined at 0, 8, 25 or 75 mg/kg/day, respectively. As shown in Table 4, skeletal variation observed at 75 mg/kg/day included the presence of 7th cervical and 14th rudimentary ribs, malaligned sternebrae, reduced ossification of the vertebral aches, and unossified sternebrae #5 or #6.

Table 4. Fetal Skeletal Variation in Dams Following Oral Administration of 2,4-D.

	Dose (mg/kg/day)			
PARAMETER	**0**	**8**	**25**	**75**
Litters Evaluated	26	29	27	26
Fetuses Evaluated	131	136	133	127
7th Cervical Rib(s)				
Fetuses # (%)	0	0	1 (1)[b]	4 (3)
Litters # (%)	0	0	1 (4)	3 (12)
14th Rudimentary Rib(s)				
Fetuses # (%)	0	1 (1)	0	4 (3)
Litters # (%)	0	1 (3)	0	3 (12)
Sternebrae malaligned				
Fetuses # (%)	7 (5)	12 (9)	11 (8)	15 (12)
Litters # (%)	7 (27)	9 (31)	8 (30)	10 (38)
Unossified Sternebrae #5/6				
Fetuses # (%)	62 (47)	62 (46)	71 (53)	73 (57)
Litters # (%)	24 (92)	27 (93)	27 (100)	22 (85)
Reduced ossification of the vertebral arch(es)				
Fetuses # (%)	2 (2)	0	3 (2)	6 (5)
Litters # (%)	1 (4)	0	3 (11)	5 (19)

a = Data obtained from Study Report Page. 30.

4. Cesarean Section Data: Reproductive data (cesarean-sectioning and litter observations) are presented in Table 3. No biologically or statistically significant effects were seen on pregnancy rate, number of implantations, litter sizes, live fetuses or dead fetuses per litter, early and rate resorptions, fetal sex ratio, crown-rump distance, or mean fetal body weights.

Table 3. Cesarean Section Findings in Pregnant Rats Given 2,4-D[a].

Observations	Dose Level [mg/kg/day]			
	0	8	25	75
# Assigned	35	35	35	35
Pregnancy Rate (%)	29(85%)	29 (85%)	28 (80%)	27 (77%)
# Nonpregnant	5 (15%)	5 (15%)	7 (20%)	8 (23%)
Maternal Wastage				
# Died	0	0	0	0
# Died pregnant	0	0	0	0
# Died nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	1	1	0	0
Total Corpora Lutea	373	351	347	337
Corpora Lutea/Dam	12.9	12.1	12.4	12.5
Total Implantations	302	286	286	272
Implantation/Dam	10.4	9.9	10.2	10.1
Total Live Fetuses	262	276	269	250
Live Fetuses/Dam	9.0	9.5	9.6	9.3
Total Dead Fetuses	0.0	0.0	0.0	0.0
Total Resorptions	40	10	17	22
Early/Dam	1.3	0.3	0.5	0.7
Late/Dam	0.0	0.1	0.1	0.1
Pre Implantation Loss (%)	19	19	18	19
Post Implantation Loss (%)	13	4	6	8
Sex Ratio (%) ♂ : ♀	58:42	53:47	57:43	57:43
Crown-Rump Length (cm)	3.6	3.6	3.6	3.6
Mean Fetal Body Wt.(g)	3.0	3.0	3.1	3.1

a = Data obtained from Study Report Pages 26, 27.

III. RESULTS

A. Maternal Toxicity

1. Mortality and Clinical Observations: No dams died at 25 or 75 mg/kg/day dose groups. Two dams delivered prematurely on gestation Day 19; one each in the control and 8 mg/kg/day groups. In both incidences, the offspring produced were of similar size and development as those from a full-term delivery. No treatment-related clinical signs of toxicity were seen; hair loss of the extremities, dried red material around the eyes and/or nares, and lacrimation were seen with similar frequency between the control and treated dams.

2. Body Weight/Body Weight Gain: Mean body weight change data are presented in Table 2. Mean body weights and body weight gain of dams at 8 and 25 mg/kg/day were comparable to those of the controls. Maternal toxicity manifested as decreases in body weight gain[a] in dams at 75 mg/kg/day during the dosing period; -43% during Days 6 through 10, -21% during Days 6 -15, and -2% during Days 0-20. Following cessation of treatment, however, body weight gain of these dams were either comparable to controls or slightly exceeded the control group for the remainder of gestation. There were no differences in mean uterine weight, net body weight (corrected body weight after removal of the uterus and contents) or in the adjusted body weight change in any of treated groups.

Table 2. Mean Body Weight Gain (g) of Rats Given Oral Administration of 2,4- D[a].

	Gestation Day				
Dose (mg/kg/day)	**0- 6**	**6 - 10**	**6 - 15**	**15- 20**	**0-20**
Vehicle Control	9	7	19	34	62
8	11(+22%)	5 (-29%)	18 (-5%)	36 (+6%)	66 (+7%)
25	9 (0%)	7 (0%)	20 (+5%)	37 (+9%)	67 (+8%)
75	9 (0%)	4 (-43%)	15 (-21%)	37 (+9%)	61 (-2%)

a = Data from Study Report Page 12.
b = value in parenthesis % difference from control.

3. Gross Pathology: No treatment-related macroscopical changes were observed either in the two dams that died at the high dose or in those sacrificed at termination.

D. DATA ANALYSIS .

1. Statistical Analysis: Maternal body weights, maternal body weight gain, mean number of corpora lutea, total implantations, viable fetuses, and fetal body weights were analyzed by ANOVA and Dunnett's test. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whittney U-test. The number of fetal sex ratios were compared by the Chi-square test with Yates correction factor. The number of litters with malformations were compared by Fisher's Exact Test.

E. REGULATORY COMPLIANCE: This study was performed in conformity with the FDA-GLP Regulations (21 CFR 58) which were in effect at the time the study was conducted (1981). A Quality Assurance Statement was dated 3/2/83.

5. Dosage Preparation and Analysis of Dosing Solutions: The test material for each group was weighed and added to a tissue grinder vessel. The vehicle was then added and the mixture was spun at a moderate speed until homogenous. Dosing solutions were prepared fresh weekly and dispensed as required, and stored at room temperature. The test material/corn oil suspension was stirred on a magnetic stirrer. Dosing solution concentrations were 2, 6.25, 18.75 ml/kg, for 8, 25 and 75 mg/kg/day dose levels, respectively Except for the analysis of the bulk material for purity and identity, no analyses of the dosing solutions were made during the study.

6. Dosage Administration: 2,4-D in corn oil was administered daily orally via gavage at doses of 0, 8 25 or 75 mg/kg/day during gestation days 6 through 15, inclusive. The vehicle control group received corn oil only. All groups received a dosing volume of 4 ml/kg body weight and the individual dosages were adjusted at each weighing interval to provide the proper mg/kg/day dose. During dosing, the dosing solution was stirred continuously using a magnetic stir plate and bars to ensure proper mixture.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: Dams were observed twice daily for general change in appearance and behavior prior to dosing, pharmacokinetics signs following dosing, and mortality/moribundity. Body weights were obtained on gestation Days 0, 6, 10, 12, 15 and 20. Maternal body weight changes during the gestation period were calculated (G-Day 20 weights minus G.Day 0 weights minus the gravid uterine weights). All surviving does were sacrificed on gestation day 20, obvious gross pathologic alterations were recorded and each gravid uterus was weighed. The thoracic, abdominal and pelvic cavities were examined for gross lesions, and in the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin. The uterus was removed from the body, examined externally, weighed and then opened for internal examination. Uteri that appeared to be from nonpregnant rats were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantations, early/late resorptions, and live/dead fetuses were recorded.

2. Fetal Examinations: Each fetus was removed from the uterus, weighed and observed for gross external alterations. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red-S, and examined for skeletal alterations. In addition, the Staples fresh dissection technique to include the heart and major vessels were used to examine the incomplete twinning observed in one fetus (#6) from dam #2371 at the high dose.

Food:	Purina Certified Rodent Chow #5002 ad libitum.
Water:	Tap water ad libitum
Environment:	Temperature, 72 ± 3°F; Humidity, 40% +; Light, 12 hr light/dark

B. PROCEDURES AND STUDY DESIGNS

1. In Life Dates - Start: 5/25/82; End: 7/22/82

2. Mating: Each female was paired with one male. Vaginal smears were taken daily during cohabitation, and the presence of copulatory plug or sperm in the vaginal smear was considered evidence of mating. The day this evidence was seen was designated as Day 0 of gestation and females were housed individually.

3. Animal Assignment: Rats were assigned, randomly, to dose groups as shown below:

Table 1. Study Design

Treatment	No. of Pregnant females	Dose Level
Control	35	0 mg/kg/day
Low Dose	35	8 mg/kg/day
Mid Dose	35	25 mg/kg/day
High Dose	35	75 mg/kg/day

4. Dose Selection Rationale: The rationale of dose level selection is based on the following: A "no effect" level of at least 25 mg/kg/day for embryo/fetotoxicity was suggested by the Pest Control Products Section, Health and Welfare Canada. In a study in Sprague-Dawley rats[a], following oral administration in corn oil at doses of 0, 12.5, 25, 50, 75 or 87.5 mg/kg/day during gestation days 6-15, inclusive, embryo/fetotoxicity was limited to the 87.5 mg/kg/day and included: decrease in fetal body weight, subcutaneous edema, delayed ossification of bone, lumber ribs and wavy ribs. Based on these data a low dose of 8 mg/kg/day was selected since it wa anticipated to be a "no effect" level. Conversely, the high dose of 75 mg/kg/day was expected to produce some degree of maternal and/or developmental toxicity.

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a = Schwetz, BA et al. 1971. *"The effect of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Esters of 2,4-D on Rat Embryonal, Foetal and Neonatal Growth and Development"*. Fd. Cosmet. Toxicol. 9:801-817

2,4-D Acid **Developmental Toxicity -Rat §83-3(a)**

I. INTRODUCTION

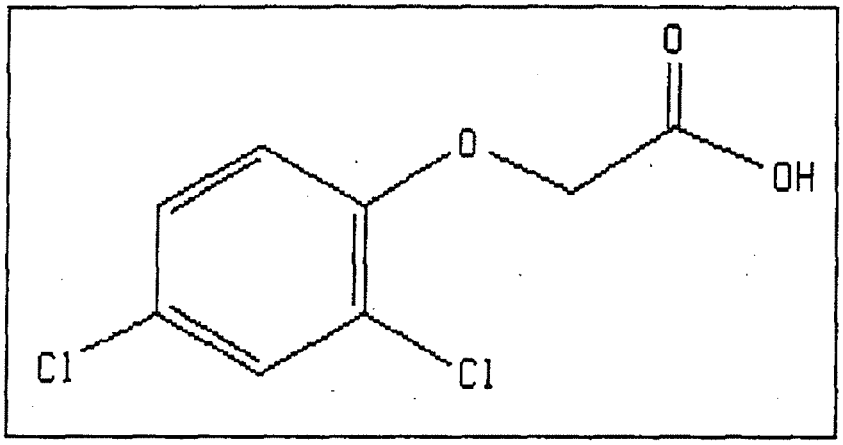
This study is re-reviewed due to the inadequacy of an earlier review (HED Doc. # 003887). The objective of this study was to assess the effects of 2,4-dichlorophenoxyacetic acid on the embryonic and fetal development following oral administration to rats during the period of organogenesis.

II. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 2,4-Dichlorophenoxyacetic acid, Technical

Description: White phenolic powder
Lot No.: 3/82
Purity: 97.%, technical
CAS #: 94-75-7
Structure:



2. Vehicle: Mazola Corn oil

3. Test Animals: Rats

Strain: Fischer 344, Charles River, Portage, MI
Age: Approximately 20 weeks old at initiation.
Weight: 173 to 238 g on Day 0 of pregnancy
Identification: Ear tags/cage cards.
Acclimation Period: Seven (7) days
Housing: Individually in stainless steel cages

2,4-D Acid **Developmental Toxicity -Rat §83-3(a)**

No treatment-related fetal gross external, visceral or skeletal malformations were seen at any dose level. Skeletal variation observed at 75 mg/kg/day included: the presence of 7th cervical ribs (4 fetuses of 3 litters vs. none in the controls); presence of 14th rudimentary ribs (4 fetuses of 3 litters vs. 0 in the controls); malaligned sternebrae (15 fetuses of 10 litters vs. 7 fetuses of 7 litters in the controls); reduced ossification of the vertebral aches (6 fetuses of 5 litters vs. 2 fetuses of 1 litter in the controls); and unossified sternebrae #5 or #6 (73 fetuses of 22 litters; 3.32/litter vs. 62 fetuses of 24 litters; 2.58/litter in the controls). Although these increases were not statistically significant, they were attributed to treatment since some of the variations (malaligned sternebrae, 14th rudimentary ribs and reduced ossification of vertebral arches) seen in this study were also seen in the F_{1b} pups of dams fed 2,4-D at 80 mg/kg/day (actual dose, ≈ 75 mg/kg/day) in the 2-generation reproduction study in the same strain of rats (Fischer 344). In addition, skeletal variations of the ribs (2nd wavy ribs, lumbar ribs) and missing sternebrae were also seen in ~~an~~ another teratology study using a different strain (Sprague-Dawley) of rats at a comparable dose of 87.5 mg/kg/day.

Thus, based upon a weight-of-evidence from the reproduction and developmental toxicity studies in Sprague-Dawley and Fischer 344 rats, it is concluded that developmental toxicity did occur at the high dose (75 mg/kg/day) in this study. **Based on these findings, for developmental toxicity, the NOEL was 25 mg/kg/day and the LOEL was 75 mg/kg/day.**

This developmental toxicity study in rats is classified as **Acceptable** and satisfies the Subdivision F Guideline requirement for a developmental toxicity study in rats [83-3(a)].